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In re: Walsh et al.
Appl. No. 09/689,430
Filed October 12, 2000

APPENDIX B

1. Get primary rat hepatocytes from Dr. Lola Reid's lab.

①. count the cells: $3.5 \times 10^6 / \text{ml}$

②. Talk with Dr. Reid and Ed.

a. special media: HDM

b. plate medium HDM + 10% FBS.

culture medium. with or without FBS.

c. Collagen I help ~~adhere~~ the cells
adhere to the bottom of dishes.

Collagen II can also induce quick differentiation
of the cells besides adhere.

d. if using collagen insert, plate the cells
in well of insert

e. plate 5×10^5 cell/well in 6-well plate

the cells will be confluent in 3-5 day
however, depending on how frequent you
feed the cells

[I found it is too much cells if
plating 5×10^5 in one well of 6-well
plate]

① 2×10^5 /well in 6-well plate.

② 5×10^5 —

③ 2×10^5 /well in 6-well plate with
Collagen I or IV insert

④ 5×10^5 /well in 6-well plate with
Collagen inserts.

for the sake of saving ~~time~~ with virus in PBS
I will use PBS.

infect. but Hepatocytes
the cells in common tissue plates adhered well.

The wells with 5×10^5 cells are almost
confluent, so I chose the 2×10^5 to use in
Common tissue 6-well plate to use

suck off the media.

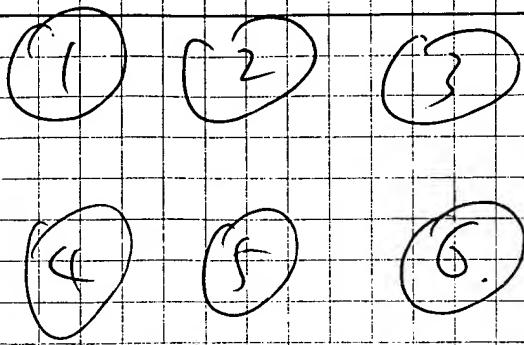
wash the wells with media.

Add the media with virus. (free of PBS)

in each well, plate at incubator for

1.5 h. swirl the plate each 15 minutes

The cells adherent to the bottom of the
well during the infection.



(1). 200 μ l VAAV/PL28

(2) round

(3) round

(4) 1 μ l

(5) 0.1 μ l

(6). 200 μ l VAAV/PL28

(200 μ l viruses sol contain about
 $2-3 \times 10^{10}$ physical particles.)

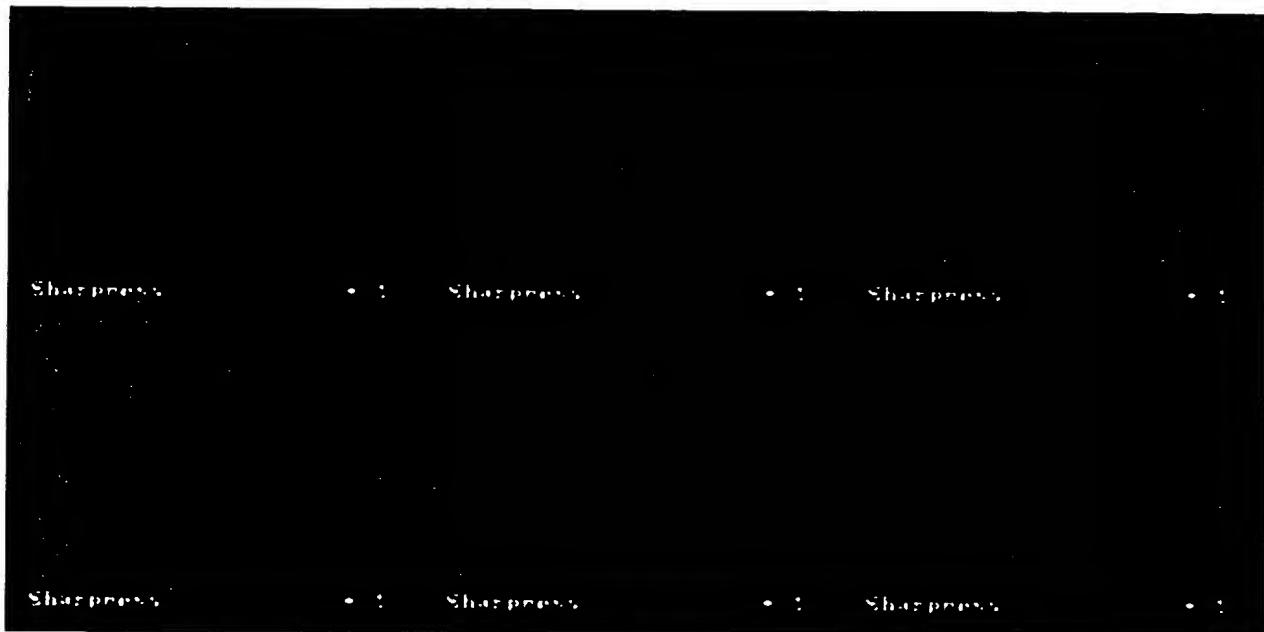
Add media (no serum) to 1 μ l of each.

④ observe the Rat-Hepatocytes / EGFP under
 UV filter with blue color. all of the cells
 even the negative control (well 6.) turned
 green.

⑤ Dr Bayned in microscopy facility confirmed
 all the cells had sth with green fluorescence
 on Monday

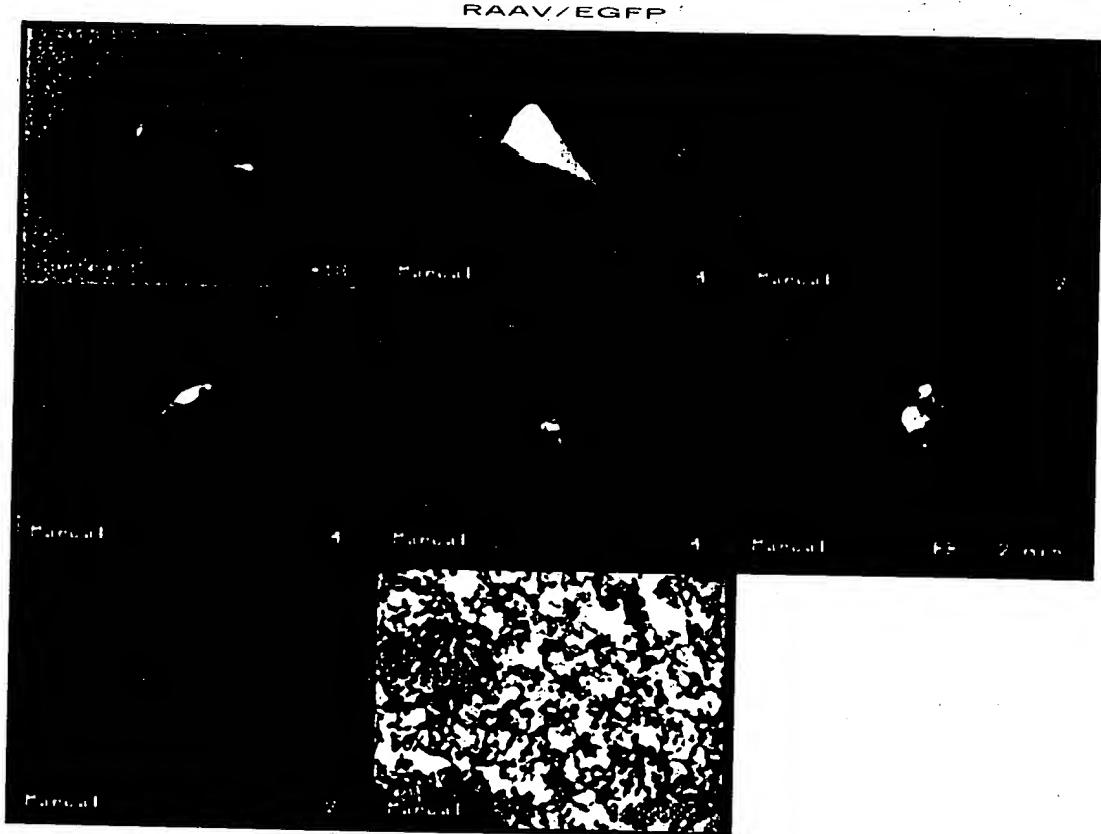
⑥ Dennis said, actually all of the primary
 Hepatocytes, Pog. human. Rat. mouse secret sth

rAAV/EGFP-Rat hepatocyte



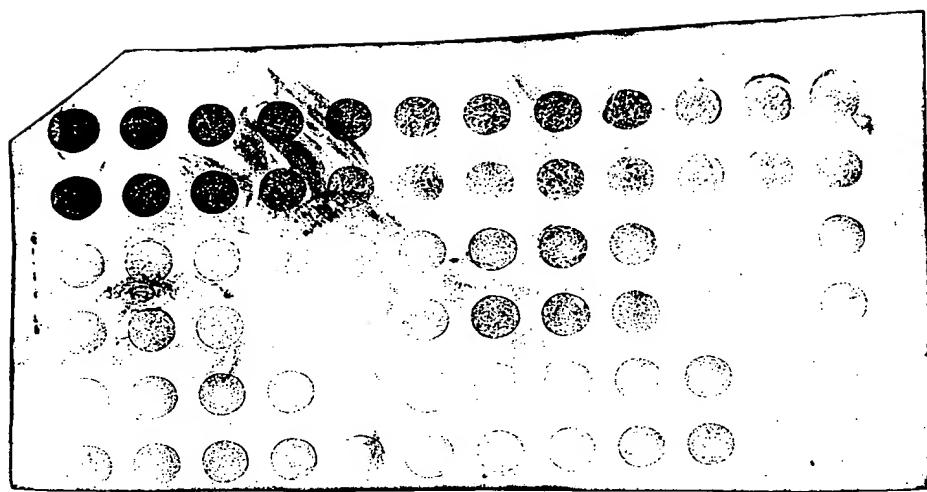
like EGFP, turn the filter to yellow
one can give some help.

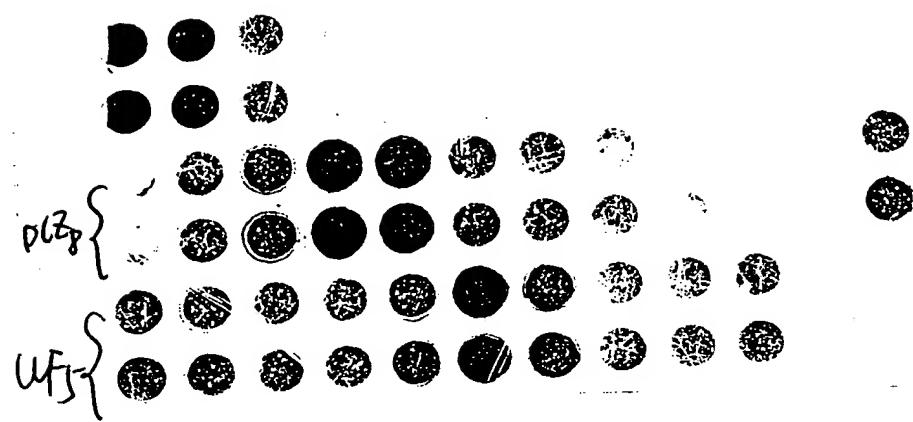
④ Did as Dennis taught
Took the pictures

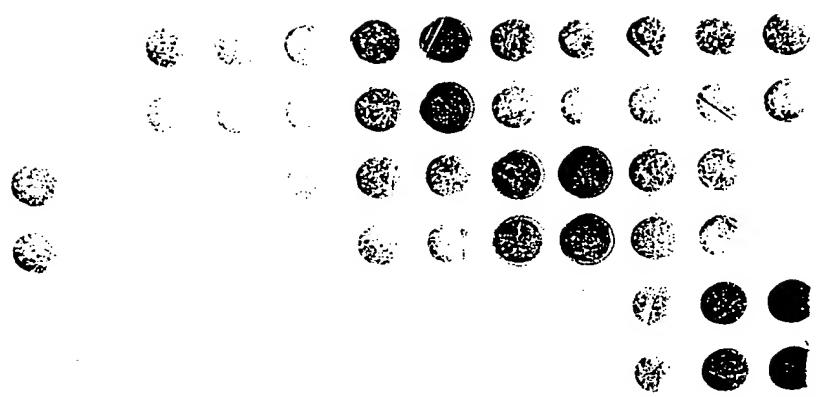


1	2	3
4	5	6
7	8	

1-6: picture from ok
cells from well 1-2
7: cells of well 6.
8: cells under common
No green cells in well 6.
few in well 4-5. light







⑤ Talk with Chris, not sure whether these green cells are Hepatocytes, instructed me to do other Hepatocyte cell lines

⑥ Coatest of Hepatocytes / DLZ6

definitely some ~~F111~~ activity from HepG2 cells
but very low

TABLE OF ABSORBANCE VALUES

DATE:

TITLE: FILE 1

AM 04:45 PM

	1	2	3	4	5	6	7	8	9	10	11	12	13
	0.0514/ml	0.024	0.012	0.006	0								
standard curve	0.445	0.453	0.466	0.289	0.211	0.146	0.046	0.045	0.046	0.045	0.044		
	0.494	0.471	0.368	0.255	0.214	0.042	0.045	0.044	0.046	0.045	0.036	0.031	
	0.301	0.237	0.28	0.195	0.210	0.137	0.239	0.184	0.047	0.045	0.045	0.046	
	0.245	0.160	0.242	0.203	0.201	0.196	0.227	0.182	0.046	0.045	0.047	0.045	
duplicate standard	0.351	0.368	0.272	0.249	0.204	0.049	0.045	0.046	0.047	0.047	0.050	0.041	
	0.358	0.306	0.291	0.259	0.216	0.047	0.046	0.045	0.047	0.047	0.048	0.044	
duplicate sample	0.222	0.205	0.214	0.214	0.223	0.112	0.224	0.221	0.046	0.047	0.047	0.045	
	0.216	0.044	0.221	0.220	0.226	0.224	0.225	0.224	0.047	0.047	0.051	0.048	
293 HepG2 WBFI44 Rat	293	day 5	day 5	day 5	primary cells	293	HepG2 WBFI44 Rat	293	day 5	day 3	293	day 5	

⑥ Apth: Day 5's sample's

HepG1F8: 96.9 / 119.4 / 109.9. HepG1O: 148.9 / >150

WBFI44/F8: 129.9. 9 > 150

293 / F8 > 150. 0 > 150

1:160 uCRP (6.3%) 108.9/10.9

1:320 uCRP (3.1%) 125.8/118.5

there are about some activity between 3-6%.

Compared to results, it is similar. The reason of the low expression of F8. perhaps are

① weak promoter, even with help of F11.

② low infectious efficiency of the viruses with the cells. (see D12.6 infecting primary rat hepatocytes)

③ In accurate titer of viruses

→ not enough viruses.

I am going to make more viruses purified by 2 times ultracentrifugation.

Re-titered by radio-immuno dot blot.